METALLOPROTEASE EXPRESSION IN ONCOGENE TRANSFORMED RODENT FIBROBLASTS.

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Metastases form after a series of sequential steps involving penetration of matrix structures and spread via the circulatory system to distant sites favourable for growth of new tumours. Metalloproteases (MMPs) are implicated in basal membrane and extracellular matrix degradation by invasive cells. The regulation of MMPs is complex and occurs at different levels: at the level of transcription, at the level of activation of proenzymes and by association with inhibitors. The genes encoding the collagenases and stromelysin metalloprotease-inhibitors. The genes encoding the collagenase and stromelysin metalloproteases have AP-1 (fos/jun) and PEA-3 transcription factor binding sites within 200 bps from the TATA elements. Transcription is stimulated by growth factors and oncoproteins such as p21-ras and pp60src. Increased stimulation of metalloprotease transcription by oncoproteins may occur during tumour progression, resulting in development of the invasive phenotype.

We and others have used normal rat embryo fibroblasts (REF) as a model to study the conversion of normal cells to invasive tumour cells. Introduction of the ras oncogene, either as a single oncogene or in unison with myc, leads to conversion of REF to metastatic tumour cells. Thus, relatively few genetic changes may be required for the induction of the metastatic phenotype. Earlier studies showed that REFs expressing activated ras genes have high activities of collagenase IV, are invasive in vitro and capable of metastasis. In contrast, cells expressing ras in unison with adenovirus E1A are not invasive and metastatic due to repression of metalloproteases expression by EIA. Recent experiments have suggested that the expression of stromelysin 1/2 correlates with the metastatic phenotype in REF cell lines expressing ras or ras + E1A (Sreenath et al., 1992, Linder et al., 1992).

It is possible that mutations in cellular genes may occur during establishment of transformed REF clones. An obvious candidate is p53. Since polyomavirus T-antigen does not bind p53, we have derived REF transformants expressing polyoma-T + ras oncogenes. Transformed cells were found to have wild-type p53 protein before and
after growth in athymic mice (by immunochemical analysis). Transformed clones capable of experimental metastasis expressed high levels of stromelysin mRNA. Surprisingly, metastatic cells showed limited *in vitro* invasion through Matrigel.

We further studied weakly metastatic clones expressing low levels of stromelysin and collagenase. Cells were injected into athymic mice and allowed to form solid tumours. Tumour derived cells showed an increased capacity for experimental metastasis, paralleled by high levels of stromelysin mRNA. Stromelysin induction occurred in the absence of increases in ras and collagenase mRNA.

Stromelysin transcription has been suggested to be dependent on binding of the AP-1 transcription factor. We have isolated a cell line which is defective in cJun, the major constituent of AP-1 (Marshall et al., 1993). Despite their lack in *c-jun* expression, these cells express stromelysin and are capable of metastasis. Taken together, available data point to the importance of stromelysin expression for metastasis of fibrosarcoma cells, and suggest that there are multiple pathways regulating stromelysin expression.

**References:**

